

REGULAR RESEARCH ARTICLE

Disruption of Long-Term Depression Potentiates Latent Inhibition: Key Role for Central Nucleus of the Amygdala

Donovan M. Ashby, Carine Dias, Lily R. Aleksandrova, Christopher G. Lapish, Yu Tian Wang, Anthony G. Phillips

Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada (Dr Ashby); Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada (Dr Dias); Department of Psychiatry, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada (Drs Aleksandrova and Phillips); Department of Psychology, Indiana University - Purdue University Indianapolis, Indianapolis, IN, United States (Dr Lapish); Department of Medicine, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada (Dr Wang).

Correspondence: Anthony G. Phillips, PhD, Department of Psychiatry, Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Room 3402 – 2215 Wesbrook Mall, Vancouver, BC, V6T 1Z3, Canada (aphillips@psych.ubc.ca).

Abstract

Background: Latent inhibition (LI) reflects an adaptive form of learning impaired in certain forms of mental illness. Glutamate receptor activity is linked to LI, but the potential role of synaptic plasticity remains unspecified.

Methods: Accordingly, the present study examined the possible role of long-term depression (LTD) in LI induced by prior exposure of rats to an auditory stimulus used subsequently as a conditional stimulus to signal a pending footshock. We employed 2 mechanistically distinct LTD inhibitors, the Tat-GluA_{2_{3Y}} peptide that blocks endocytosis of the GluA2-containing glutamate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, or the selective glutamate n-methyl-d-aspartate receptor 2B antagonist, Ro25-6981, administered prior to the acquisition of 2-way conditioned avoidance with or without tone pre-exposure.

Results: Systemic LTD blockade with the Tat-GluA_{2_{3Y}} peptide strengthened the LI effect by further impairing acquisition of conditioned avoidance in conditional stimulus-preexposed rats compared with normal conditioning in non-preexposed controls. Systemic Ro25-6981 had no significant effects. Brain region-specific microinjections of the Tat-GluA_{2_{3Y}} peptide into the nucleus accumbens, medial prefrontal cortex, or central or basolateral amygdala demonstrated that disruption of glutamate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor endocytosis in the central amygdala also potentiated the LI effect.

Conclusions: These data revealed a previously unknown role for central amygdala LTD in LI as a key mediator of cognitive flexibility required to respond to previously irrelevant stimuli that acquire significance through reinforcement. The findings may have relevance both for our mechanistic understanding of LI and its alteration in disease states such as schizophrenia, while further elucidating the role of LTD in learning and memory.

Keywords: Synaptic plasticity, cognitive flexibility, long-term depression (LTD), central amygdala (CeA), latent inhibition (LI)

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Significance Statement

Latent inhibition (LI) is a learning phenomenon whereby prior repeated exposure to inconsequential stimuli disrupts subsequent conditioning of such stimuli with reinforcement. Glutamate receptor activity is linked to LI, but the potential role of synaptic plasticity remains unspecified. Systemic disruption of long-term depression (LTD) using an interference peptide to inhibit AMPA receptor endocytosis strengthened the LI effect by further impairing avoidance learning in rats pre-exposed to the tone stimulus compared with the non-preexposed condition. Targeted peptide microinjections to disrupt LTD, specifically in the central amygdala, replicated this potentiated LI effect. These findings are relevant to understanding the neural circuits underlying LI as well as the role of synaptic LTD in learning and memory. Given the prominence of aberrant LI in schizophrenia, these data may also have clinical relevance.

Introduction

The flexible and efficient allocation of cognitive resources to guide behavior in a changing environment is important for survival. Latent inhibition (LI) is a learning phenomenon where repeated exposure to an inconsequential stimulus impairs the subsequent conditioning of this stimulus with reinforcement (Lubow, 1973). While learning to ignore irrelevant stimuli is considered an adaptive mechanism, pathological alteration of LI is associated with cognitive dysfunction in schizophrenia (Lubow and Weiner, 2010a).

The acquisition-failure theory of LI attributes the retarded conditioning to the pre-exposed stimulus to decreased associability (Lubow et al., 1976) or its salience (Pearce and Hall, 1980). Alternatively, the competition theory emphasizes opposing processes between 2 conflicting associations, specifically conditional stimulus (CS)-no event acquired in pre-exposure and CS-US acquired subsequently during conditioning, which compete for behavioral expression/retrieval (Weiner, 1990). Reconciliation of these 2 approaches postulates that CS pre-exposure attenuates subsequent acquisition of the CS-event association, which in turn competes with the original CS-no event association (Lubow and Weiner, 2010b). Behavioral, physiological, and pharmacological manipulations can promote or impair switching to respond according to the stimulus reinforcement association and thereby weaken or strengthen the expression of LI (Weiner and Feldon, 1997; Weiner, 2003). Many of these accounts implicate changes in synaptic plasticity in different learning processes during LI, but there are no direct tests of this hypothesis.

Descriptions of the neural underpinnings of LI traditionally focus on the nucleus accumbens (NAc) and the action of dopamine therein (Weiner and Arad, 2009). The switching model of LI (Weiner and Feldon, 1997), which implicates NAc circuits in resolving conflicts arising from 2 competing associations (CS-event/no event), is supported by an extensive literature on both the modulatory effects of dopaminergic drugs (Weiner et al., 1996; Schiller et al., 2006) and NAc lesions on aspects of LI (Weiner et al., 1996, 1999; Jongen-Rêlo et al., 2002; Pothuizen et al., 2005; Floresco, 2015). These studies show that lesions of the NAc shell can increase, whereas NAc core lesions can decrease, flexibility in LI (Weiner et al., 1996; Jongen-Rêlo et al., 2002; Gal et al., 2005). In addition, the dopamine releaser amphetamine, which can produce/exacerbate psychotic-like symptoms in both animals and humans, disrupts LI at the conditioning stage, while typical and atypical antipsychotic drugs, scopolamine, and glutamate n-methyl-d-aspartate receptors (NMDAR) antagonists (phencyclidine, ketamine, and MK-801) produce persistent LI also via effects at conditioning (Gaisler-Salomon and Weiner, 2003; Weiner, 2003; Gaisler-Salomon et al., 2008).

The use of specific inhibitors of synaptic plasticity provides an effective strategy for delineating detailed synaptic mechanisms underlying different learning paradigms (Citri and

Malenka, 2008; Howland and Wang, 2008; Collingridge et al., 2010; Takeuchi et al., 2014). Long-term depression (LTD) is a form of synaptic plasticity involving activity-dependent weakening of excitatory neurotransmission at glutamate synapses. LTD is implicated in mediating cognitive and behavioral flexibility in tasks that involve a change in reinforcement contingencies, whereby previously acquired contingencies disrupt acquisition of behavioral responding guided by the current contingencies. Our group and others have shown that blocking LTD prior to conditioning impairs fear extinction (Dalton et al., 2008, 2012), spatial reversal learning (Kim et al., 2011; Dong et al., 2013), and natural forgetting (Hardt et al., 2014; Míguas et al., 2016).

Based on these previous findings, we reasoned that LTD may participate in the changing reinforcement contingencies in stimulus pre-exposed animals. In accordance with the competition theory (Weiner, 1990; Weiner and Feldon, 1997), we hypothesized that disruption of LTD prior to conditioning in an LI paradigm would have a selective detrimental effect on avoidance learning in rats pre-exposed to the CS by interfering with the cognitive flexibility required to overwrite a stimulus-no response association while having no effect in the non-preexposed (NPE) group where no prior association had been acquired. In the present study, LTD was blocked by either disrupting clathrin-dependent endocytosis of GluA2-containing α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), the critical final step in LTD expression, using the Tat-GluA₂_{3Y} peptide (Ahmadian et al., 2004; Brebner et al., 2005; Dalton et al., 2008, 2012), or by inhibiting GluN2B subunit-containing NMDARs implicated in the initiation of LTD, using the selective antagonist Ro25-6981 (Liu et al., 2004). Initially, both LTD manipulations were administered systemically to examine the potential role of LTD in modulating LI of acquisition of 2-way active avoidance of a footshock predicted by an auditory tone stimulus. To further identify key brain regions where inhibition of AMPAR endocytosis may mediate effects of LI, the Tat-GluA₂_{3Y} peptide was microinjected into the NAc and medial prefrontal cortex (mPFC), a key afferent projection to the NAc (George et al., 2010; Lingawi et al., 2016). Given our use of an avoidance paradigm to assess LI, we also included the central nucleus of the amygdala (CeA) due to its roles in active defensive responses (Tillman et al., 2018) and the control of dopaminergic activity during appetitive and aversive learning (Ahn and Phillips, 2002; Steinberg et al., 2020). A less well-known function of the CeA is in allocating attention resources to cues when their predictive value changes (Holland and Gallagher, 1993a, 2006; Lee et al., 2006). Extending this capacity to LI suggests the CeA may play a similar role when familiar cues with minimal predictive value suddenly gain significance through reinforcement.

Methods and Materials

Subjects

Male Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 200–220 g on arrival were pair-housed in a colony room (temperature: 21°C±1°C) under a reverse light cycle (light off 7:00 AM–7:00 PM). Food and water were available ad libitum. After the i.v. surgery, the animals were isolated for 3 days to ensure that the surgical wound on their back was sufficiently healed before being housed again with their cage mate. All experiments followed the principles of laboratory animal care and were conducted in accordance with the standards of the Canadian Council on Animal Care and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003). All the experiments were approved by the Committee on Animal Care, University of British Columbia.

Surgery

Implantation of i.v. catheter

Rats were anaesthetized with isoflurane (oxygen flow rate: 2 L/min; isoflurane: induction: 4%; maintenance: 1.5–2.5%; Baxter Corporation, Canada) and an indwelling silastic catheter (Dow Corning Corporation, US) was implanted with the proximal end inserted into the right jugular vein. The distal end was connected to a plastic screw-on connector (Plastics-One Inc., Roanoke, VA, USA) mounted on a square mesh (Plastics-One Inc., US) and secured by dental cement. The catheter was located s.c. and the plastic connector exited through an incision between the scapulae. The i.v. catheter was flushed daily with a heparinized (30 IU/mL; LEO Pharma Inc., Canada) ampicillin solution (0.2 mL of 50 mg/mL; Novopharm, Canada) to prevent infection and keep the catheter patent.

Intracranial surgery

Rats were anaesthetized with isoflurane (oxygen flow rate: 2 L/min; isoflurane: induction: 4%; maintenance: 1.5–2.5%) and mounted in a Kopf stereotaxic frame with the incisor bar set at 3.3 mm below the interaural line. Bilateral guide cannulas (23-gauge, Small Parts Inc. Miami Lake) were implanted, aimed 1.5 mm above the NAc (anteroposterior [AP]: +1.65, mediolateral [ML]: ±1.2, dorsoventral [DV] –5.5), 1.5 mm above the central amygdala (AP: –1.9; ML: ±4, DV: –6.7), 1.5 mm above the basolateral amygdala (AP: –2.1, ML: ±4.8, DV: –6.8), or 0.5 mm above the mPFC (AP: +2.1, ML: ±0.8, DV: –2.2). AP, ML, and DV coordinates were calculated relative to Bregma (Paxinos and Watson, 2013). Injection needles extended 1.5 mm beyond the guide cannula tip for NAc, CeA, and basolateral amygdala (BLA) experiments and 0.5 mm beyond the guide cannula tip for mPFC experiments. The cannulas were anchored to the skull with dental acrylic cement (Simplex; Kent Dental Supplies, Gillingham, Kent, UK) and 4 surgical screws. Stainless-steel obturators (30-gauge, Small Parts Inc.) were inserted in the cannula to prevent occlusion.

LI Protocol

Each avoidance apparatus (41.8×25.5×16.2 cm; Med Associates, St. Albans, VT) is comprised of 2 compartments of equal dimension separated by a guillotine door. Each compartment was equipped with 4 photocells, a wall-mounted cue light, and a white noise generator (approximately 60 dB) on each rear end. The LI protocol consisted of 2 different phases. On day 1 and 2,

there were 2 groups of animals pre-exposed to the test apparatus along with either: (a) the presentation of fifty 10-second conditioned stimuli (CS: white noise presented at pseudo-random intervals (range 10–120 seconds, mean: 60 seconds) (pre-exposed rats, PE), or (b) the absence of auditory stimuli (NPE). Each session lasted 60 minutes (15 minutes habituation + 45 minutes session). On day 3, 2-way avoidance training began with a 15-minute habituation period followed by 100 CS-unconditioned stimuli footshock pairing trials presented at pseudo-random intervals. The auditory CS (10 seconds) was identical to the tone presented previously to the PE group. A footshock (2 seconds, 0.75 mA) was delivered at the end of the CS. An avoidance response was defined by movement into the opposite compartment prior to CS termination. Escape responses were defined as entry into the opposite compartment during the 2-second footshock. Movement after footshock termination constituted a response failure. The main dependent variable was the number of avoidance responses recorded during the 100-trial session.

Drugs

The selective GluN2B subunit antagonist Ro25-6981 (Sigma-Aldrich, Oakville, Canada) was dissolved in the vehicle (2% DMSO and 0.09% isotonic saline). The Tat-GluA2_{3Y} peptide was constituted of 9 amino acids (YKEGYNVYGY) (Ahmadian et al., 2004) and was attached to a HIV-1-derived Tat peptide sequence (YGRKKRRQRRR) to cross the blood brain barrier and permeate cells (Schwarze et al., 1999). The scrambled peptide Tat-GluA2_{sc} was comprised of a scrambled sequence of the same 9 amino acids (VYKYGGYNE). Both Tat-GluA2_{3Y} and Tat-GluA2_{sc} were synthesized in the Wang laboratory. These peptides were diluted in 0.9% sterile saline for the i.v. administration and in phosphate buffer saline (0.05 M) for the intracerebral administration.

Drug Treatment

All drug treatments were administered exclusively on day 3 prior to the initiation of 2-way avoidance training. Control assessment of LI (NPE: n=6; PE: n=7) involved i.v. administration of saline 45 minutes prior the onset of avoidance training. A similar protocol was used for AMPAR endocytosis inhibitor experiments, with Tat-GluA2_{3Y} (2.25 nmol/g; NPE: n=7, PE: n=7) or the control peptide, Tat-GluA2_{sc} (2.25 nmol/g; NPE: n=12, PE: n=13) administered i.v. 45 minutes prior to starting the training session. GluN2B antagonist experiments involved i.p. injection of 6 mg/kg of Ro25-6981 (NPE: n=6, PE: n=12) or vehicle (NPE: n=8, PE: n=7) 15 minutes before placement into the apparatus. During intracerebral microinjection experiments, injection needles (33-gauge, Small Parts Inc.) were inserted into guide cannulae. Tat-GluA2_{3Y} or Tat-GluA2_{sc} (22.5 pmol/0.5 µL) was administered at a rate of 0.5 µL/min. At the end of the injection, the needles were left in place for 2 minutes to ensure diffusion and were then replaced by obturators. Animals received bilateral injections of 0.5 µL/side into the NAc (Tat-GluA2_{3Y} NPE: n=7, PE: n=7; Tat-GluA2_{sc} NPE: n=6, PE: n=8), 0.75 µL/side into the mPFC (Tat-GluA2_{3Y} NPE: n=5, PE: n=7; Tat-GluA2_{sc} NPE: n=5, PE: n=6), and 0.5 µL/side into the CeA (Tat-GluA2_{3Y} NPE: n=6, PE: n=8; Tat-GluA2_{sc} NPE: n=7, PE: n=7). To exclude possible effects of intra-CeA Tat-GluA2_{3Y} spreading into the BLA, 2 additional groups (NPE: n=8, PE: n=9) were tested on acquisition of 2-way avoidance following bilateral injections of Tat-GluA2_{3Y} (0.5 µL/side) into the BLA and compared with Tat-GluA2_{3Y} CeA treatment groups.

Histology

After completion of each experiment, animals were deeply anesthetized with isoflurane and brains were removed and stored in 20% w/v sucrose and 4% v/v paraformaldehyde solution for at least 48 hours. Coronal sections (30 μ m) were stained with Cresyl violet (Fisher Scientific, Ottawa, Canada) and examined for injection site location ([supplementary Figure 1](#)).

Statistical Analysis

The avoidance responses were analyzed using a repeated-measures mixed general linear model (GLM) using SPSS (IBM), with 10-trial bins as the within-subject variable and exposure and drug as between-subject fixed effects. Acquisition of 2-way avoidance was indicated by a main effect of trial bins, whereas LI was indicated by a main effect of exposure. To assess the impact of drug interventions (Ro25-6981 vs vehicle, Tat-GluA_{2_{3y}} vs Tat-GluA_{2_{sc}}) on LI, a significant drug by exposure interaction was followed by simple main effects comparisons (SME) between drug treatment groups in PE and NPE

groups, respectively, with Bonferroni correction for multiple comparisons. Escape responses were analyzed in experiments with a significant group effect on avoidance to confirm that these effects were not explainable by a nonspecific effect on locomotor activity. Differences were considered significant when $P < .05$.

Results

As expected, there was a significant difference in 2-way avoidance acquisition between vehicle-treated PE and NPE rats, reflecting LI. Pre-PE to the CS slowed learning of avoidance responding to subsequent CS-US pairing compared with the NPE condition ([Figure 1A, B](#); 2-way mixed GLM, main effect of trials $F(9,99) = 6.345$, $P < .001$; main effect of exposure $F(1,11) = 8.252$, $P = .015$; trials by exposure interaction $F(9,99) = 3.868$, $P < .001$). Escape responses declined progressively over trials, with fewer escape responses in NPE vs PE rats ([Figure 1C, D](#); main effect of trials $F(9,99) = 5.739$, $P < .001$; main effect of exposure $F(1,11) = 8.408$, $P = .014$; trials by exposure interaction $F(9,99) = 3.752$, $P < .001$).

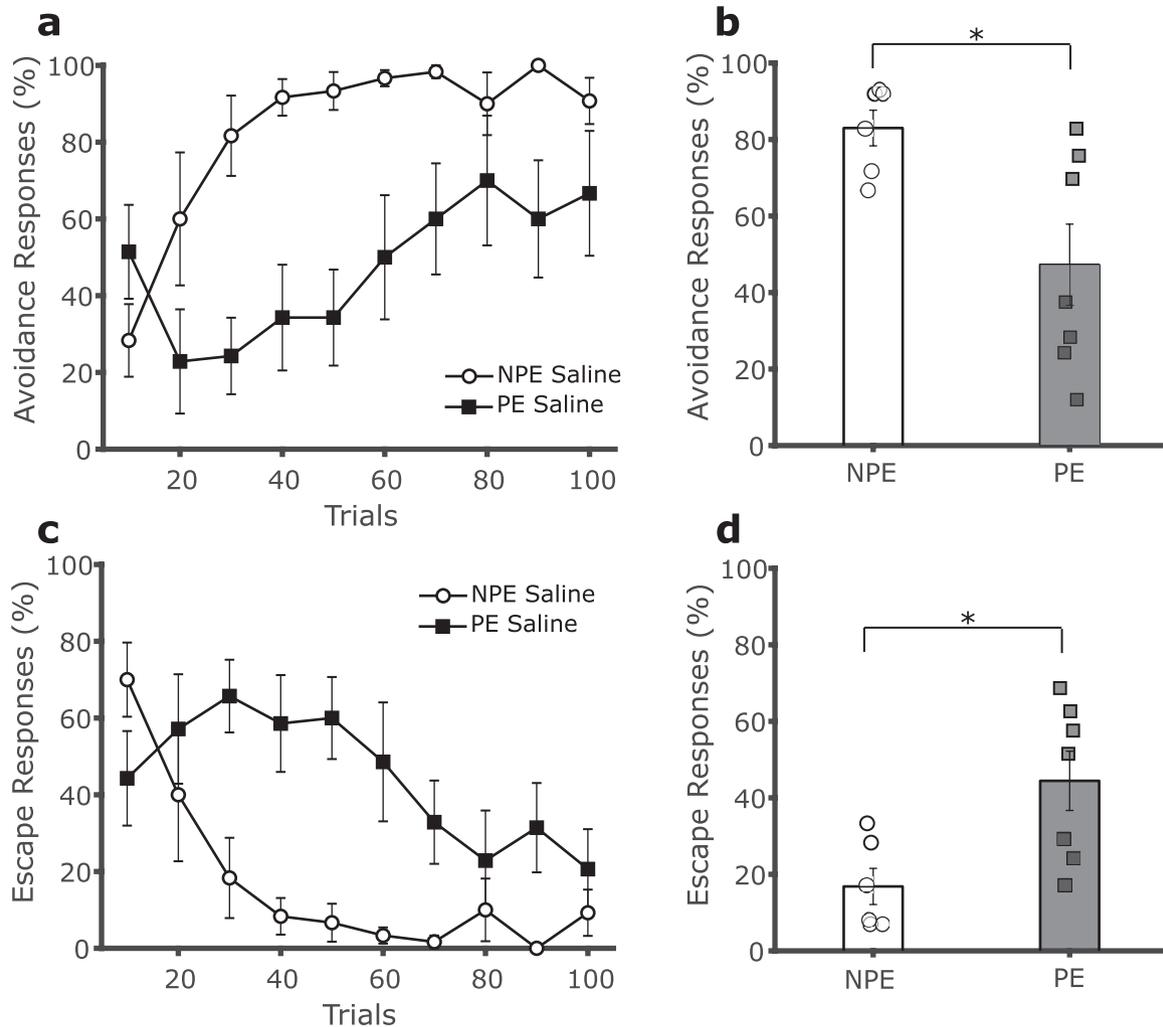


Figure 1. Latent inhibition (LI) of 2-way avoidance. (A) Strong LI was observed in our paradigm, as pre-exposure (PE, filled squares) to the CS significantly impaired acquisition of avoidance responding relative to non-preexposed (NPE, empty circles) rats, as observed in 10 trial bins. Successful avoidance responses involved shuttling to the opposite side of the avoidance chamber during the 10-second tone presentation, which predicted a 2-second footshock. (B) Overall avoidance percentage across 100 trials was significantly lower in PE vs NPE rats, reflecting LI. (C) Escape responses declined progressively over trials, with fewer escape responses in NPE vs PE rats. (D) Overall escape responses percentage across 100 trials was significantly lower in PE vs NPE rats. * $P < .05$, error bars represent SEM.

Systemic administration of the AMPAR endocytosis interference peptide Tat-GluA_{23Y} prior to conditioning further significantly reduced avoidance learning in PE rats relative to PE rats treated with a scrambled control peptide. Importantly, Tat-GluA_{23Y} treatment had no measurable effect on performance in NPE rats, resulting in a much stronger LI effect (i.e., larger difference between PE and NPE groups) in the Tat-GluA_{23Y} peptide condition (Figure 2A, B; 3-way mixed GLM, main effect of trials $F(9,315) = 24.617, P < .001$; main effect of exposure $F(1,35) = 25.059, P < .001$; main effect of drug $F(1,35) = 4.256, P = .047$; exposure by drug interaction $F(1,35) = 4.365, P = .044$; follow-up SME of drug in NPE $F(1,33) = 0.001, P = .985$; follow-up SME of drug in PE $F(1,35) = 8.746, P = .006$). Escape responses progressively declined over trials (Figure 2C, D; $F(9,315) = 16.802, P < .001$) with fewer escapes in NPE vs PE rats ($F(1,35) = 13.580, P = .001$), although this pattern was not seen in Tat-GluA_{23Y}-treated PE rats. No significant effect of Tat-GluA_{23Y} treatment or interaction was observed

(main effect of drug $F(1,35) = 0.208, P = .651$; exposure by drug interaction $F(1,35) = 1.098, P = .302$).

To investigate the specific role of the NMDAR GluN2B subunit implicated in the induction of LTD, we systemically administered the GluN2B subunit-specific antagonist Ro25-6981 prior to conditioning. GluN2B receptor antagonism did not affect acquisition of 2-way avoidance in PE or NPE rats, indicating no significant effect of Ro25-6981 on LI (Figure 3A, B; 3-way mixed GLM, main effect of trials $F(9,261) = 35.361, P < .001$; main effect of exposure $F(1,29) = 17.375, P < .001$; main effect of drug $F(1,29) = 0.274, P = .604$; exposure by drug interaction $F(1,29) = 1.470, P = .235$). Thus, blocking GluN2B-dependent NMDAR signaling did not recapitulate the effect observed with the Tat-GluA_{23Y} peptide. As before, escape responses declined over trials, with fewer escapes in NPE vs PE rats, with no effect of Ro25-6981 treatment (data not shown, main effect of trials $F(9,261) = 22.375, P < .001$; main effect of exposure $F(1,29) = 8.062,$

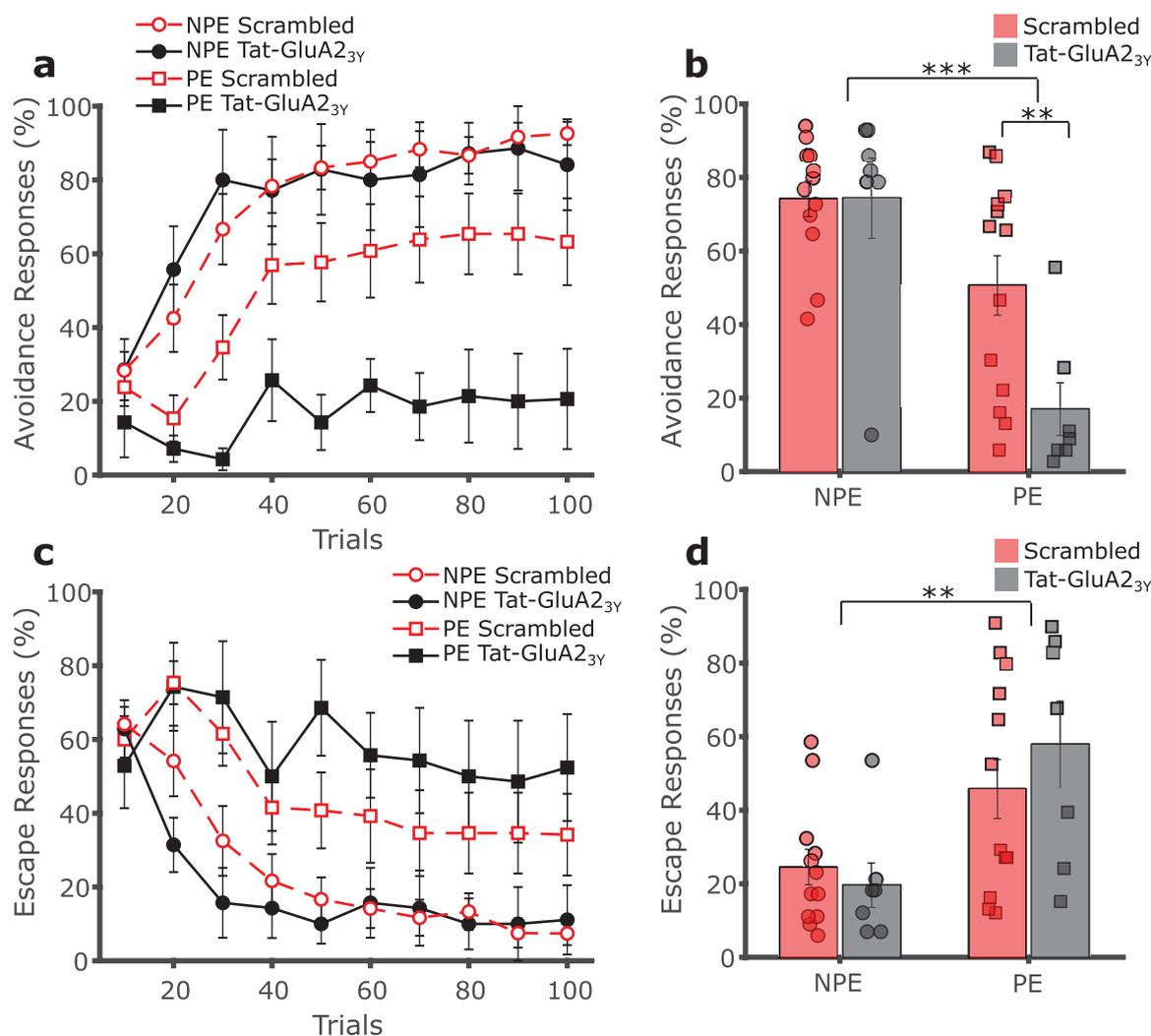


Figure 2. Systemic Tat-GluA_{23Y} potentiates latent inhibition (LI) of 2-way avoidance. (A) The AMPA receptor endocytosis inhibitor Tat-GluA_{23Y} (2.25 nmol/g, i.v., black, solid lines) was administered systemically to rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls. Tat-GluA_{23Y} markedly strengthened the LI effect by further impairing avoidance responding in PE rats (squares) relative to rats administered a scrambled control peptide (red, dashed lines), while having no effect in the NPE group (circles). (B) Overall avoidance percentage indicated a significant reduction of avoidances in Tat-GluA_{23Y}-treated PE rats compared with the NPE group and the corresponding scrambled control, indicating a potentiation of LI. (C) Escape responses progressively declined over trials, with fewer escapes in NPE vs PE rats, although this was not observed in the PE GluA_{23Y} group. No effect of GluA_{23Y} treatment or interaction was observed. (D) Overall escape responses percentage was significantly lower in PE vs NPE rats, and no effect of GluA_{23Y} treatment or interaction was observed. ** $P < .01$, *** $P < .001$, error bars represent SEM.

$P = .008$; main effect of drug $F(1,29) = 2.042$, $P = .164$; exposure by drug interaction $F(1,29) = 0.418$, $P = .523$).

To determine a locus of action for the observed effect of systemic Tat-GluA_{23Y} peptide administration, separate experiments were conducted in which the interference peptide was administered prior to conditioning via intracerebral microinjection to several key brain regions previously implicated in LI. Intra-Nac administration of the Tat-GluA_{23Y} peptide did not alter the expression of LI relative to the control peptide (Figure 4A, B; 3-way mixed GLM, main effect of trials $F(9,216) = 29.043$, $P < .001$; main effect of exposure $F(1,24) = 5.057$, $P = .034$; main effect of drug $F(1,24) = 0.004$, $P = .951$; exposure by drug interaction $F(1,24) = 0.016$, $P = .901$). Similarly, intra-mPFC administration of the Tat-GluA_{23Y} peptide did not affect expression of LI relative to the control peptide (Figure 5A, B; 3-way mixed GLM,

main effect of trials $F(9,171) = 10.494$, $P < .001$; main effect of exposure $F(1,19) = 10.857$, $P = .004$; main effect of drug $F(1,19) = 0.200$, $P = .660$; exposure by drug interaction $F(1,19) = 0.114$, $P = .740$).

Microinjection of Tat-GluA_{23Y} peptide into the CeA significantly potentiated LI relative to the control peptide, as indicated by a profound impairment in the acquisition of 2-way avoidance in PE rats, compared with intact conditioning in their NPE counterparts and control PE rats (Figure 6A, B; 3-way mixed GLM, main effect of trials $F(9,216) = 27.351$, $P < .001$; main effect of exposure $F(1,24) = 39.223$, $P < .001$; main effect of drug $F(1,24) = 11.152$, $P = .003$; exposure by drug interaction $F(1,24) = 4.375$, $P = .047$; follow-up SME of drug in NPE $F(1,24) = 0.726$, $P = .403$; follow-up SME of drug in PE $F(1,24) = 15.896$, $P = .001$). Escapes declined progressively over time with fewer escapes in NPE vs PE rats (Figure 6C, D; main

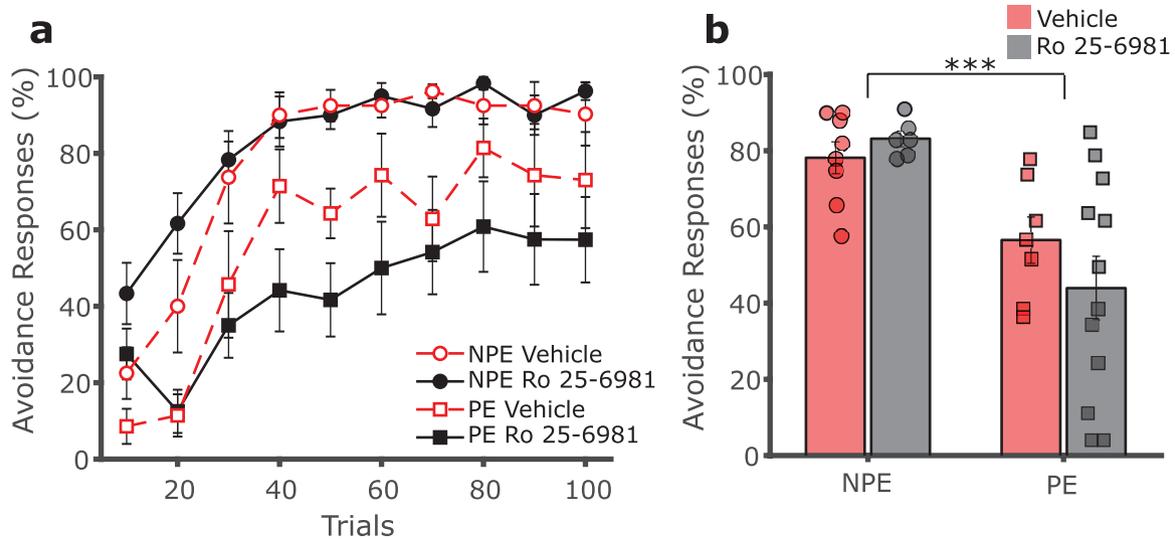


Figure 3. Ro25-6981 does not affect latent inhibition of 2-way avoidance. (A) The GluN2B receptor antagonist Ro25-6981 (6 mg/kg, i.p., black, solid lines) or vehicle (red, dashed lines) was administered systemically to rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls irrespective of the drug treatment, with no effect on LI. (B) Overall avoidance percentage was significantly lower in PE vs NPE rats, with no effect of drug treatment. *** $P < .001$, error bars represent SEM.

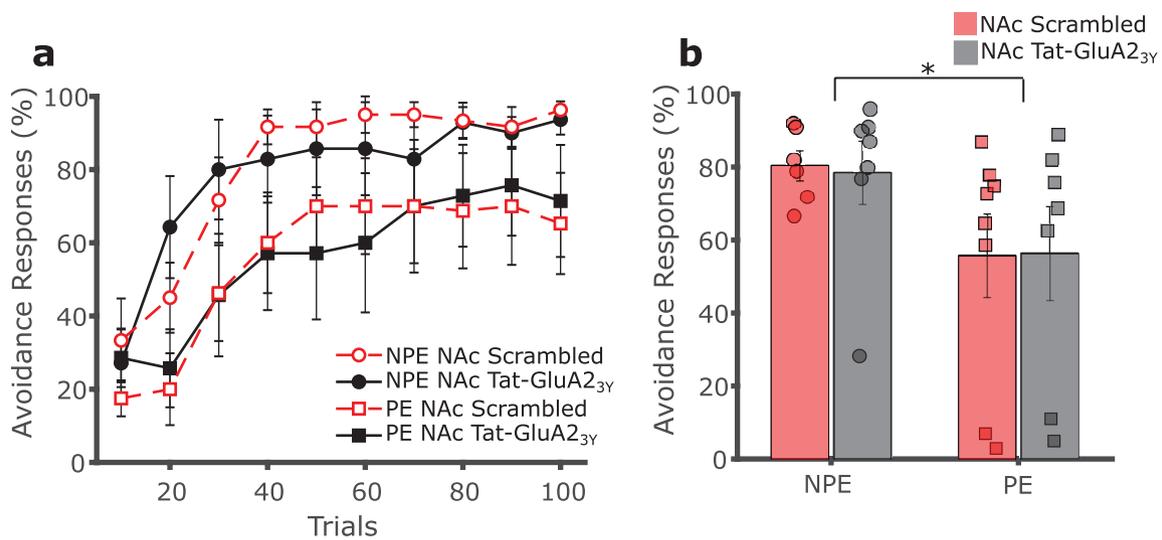


Figure 4. Nucleus accumbens (Nac) administration of Tat-GluA_{23Y} does not affect latent inhibition (LI) of 2-way avoidance. (A) Tat-GluA_{23Y} (0.5 μ L/hemisphere, 22.5 pmol/0.5 μ L, IC, black, solid lines) or scrambled control peptide (red, dashed lines) was administered into the Nac of rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls irrespective of the drug treatment, with no effect on LI. (B) Overall avoidance percentage was significantly lower in PE vs NPE rats, with no effect of drug treatment. * $P < .05$, error bars represent SEM.

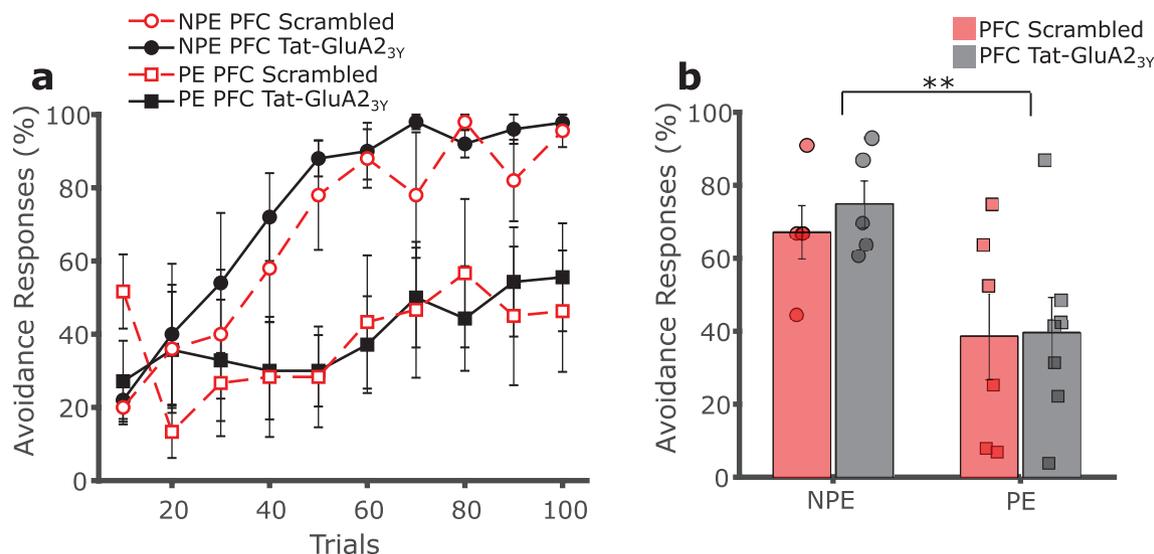


Figure 5. Medial prefrontal cortex (mPFC) administration of Tat-GluA23Y does not affect latent inhibition (LI) of 2-way avoidance. (A) Tat-GluA23Y (1.0 μ L/hemisphere, 22.5 pmol/0.5 μ L, IC, black, solid lines) or scrambled control peptide (red, dashed lines) was administered into the mPFC of rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls irrespective of the drug treatment, with no effect on LI. (B) Overall avoidance percentage was significantly lower in PE vs NPE rats, with no effect of drug treatment. ** $P < .01$, error bars represent SEM.

effect of trials $F(9,216) = 13.877$, $P < .001$; main effect of exposure $F(1,24) = 40.996$, $P < .001$, while Tat-GluA_{23Y} treatment tended to elevate escapes in all groups, indicating that drug treatment did not impair perception of a noxious stimulus or locomotor activity (main effect of drug $F(1,24) = 9.139$, $P = .006$; exposure by drug interaction $F(1,24) = 3.860$, $P = .061$).

To confirm this LI effect was mediated by drug action in the CeA and not due to spill over into the BLA, the Tat-GluA_{23Y} peptide was administered directly into the BLA prior to conditioning in separate groups of PE and NPE rats. BLA administration of Tat-GluA_{23Y} did not recapitulate the significant impairment of avoidance learning following CeA administration of Tat-GluA_{23Y}, confirming the CeA as the locus of action for the peptide (Figure 7; 3-way mixed GLM, main effect of trials $F(9,243) = 19.200$, $P < .001$; main effect of exposure $F(1,27) = 27.396$, $P < .001$; main effect of region $F(1,27) = 4.459$, $P = .044$; exposure by region interaction $F(1,27) = 5.363$, $P = .028$; follow-up SME of region in NPE $F(1,27) = 0.019$, $P = .892$; follow-up SME of region in PE $F(1,27) = 10.954$, $P = .003$).

Discussion

As expected, pre-exposure to a nonreinforced stimulus impaired subsequent conditioning of this stimulus with reinforcement compared with the non-preexposed condition. The present LI paradigm involved strong CS pre-exposure (100 CS presentations over 2 days) and therefore produced robust LI. Although avoidance conditioning in PE rats was significantly lower than in NPE rats throughout the 100 trials, PE rats still displayed a learning curve. In stark contrast, systemic administration of the Tat-GluA_{23Y} peptide completely blocked avoidance conditioning in PE rats, with no apparent learning curve. The Tat-GluA_{23Y} peptide has been extensively validated in vivo across multiple brain regions as a specific inhibitor of clathrin-dependent endocytosis of GluA2-containing AMPARs, a critical step in LTD expression with no effect on basal synaptic transmission or LTP (Ahmadian et al., 2004; Brebner et al., 2005; Dalton et al., 2008; Yu et al., 2008; Ge et al., 2010; Dong et al., 2013).

Our results indicate that blocking LTD expression during the conditioning phase of a 2-way avoidance procedure potentiates LI by impairing avoidance learning to a pre-exposed CS. The absence of any disruptive effect of LTD blockade on conditioning with a novel CS in NPE rats excludes the possibility that this effect is due to a general impairment of associative learning per se or the capacity to detect and protect from aversive stimuli. This is consistent with intact fear conditioning under LTD blockade (Dalton et al., 2008, 2012) and extends this finding to active fear-conditioned avoidance. Thus, peptide-mediated LTD blockade during conditioning resulted in behavioral perseveration in the LI paradigm or an impaired capacity of PE rats to switch responding from the initial CS-no event association to the CS-US association, consistent with a role for LTD in mediating behavioral flexibility in response to changes in reinforcement contingency. Similarly, in previous studies of LTD blockade at the conditioning stage, fear conditioning is acquired but not extinguished (Kim et al., 2007; Dalton et al., 2008, 2012), and the initial hidden platform location on a water maze, but not its relocation, is learned (Nicholls et al., 2008; Kim et al., 2011; Dong et al., 2013). LTD is also required for natural forgetting of previous associations (Hardt et al., 2014; Míguas et al., 2016).

Systemic blockade of LTD using the Tat-GluA_{23Y} peptide produces similar effects on LI perseveration as dopamine blockers, NMDA antagonists, and cholinergic drugs (Weiner, 2003). As these drugs have known effects on synaptic plasticity, LTD represents a potential common mechanism by which such drugs may mediate their effect on LI, although other mechanisms may also be involved. Here we report significant treatment-induced strengthening of LI compared with strong LI in controls, whereas previous demonstrations of LI enhancement often involved weak or no LI in controls produced with fewer CS pre-exposure (Weiner, 2003), underscoring the robustness of our LTD blockade effect. As Tat-GluA_{23Y} treatment entirely blocked conditioning in PE rats, an interesting question remains as to the effects of weaker pre-exposure protocols followed by LTD blockade in conditioning.

On the other hand, systemic administration of the GluN2B subunit-selective NMDAR antagonist, Ro25-6981, which

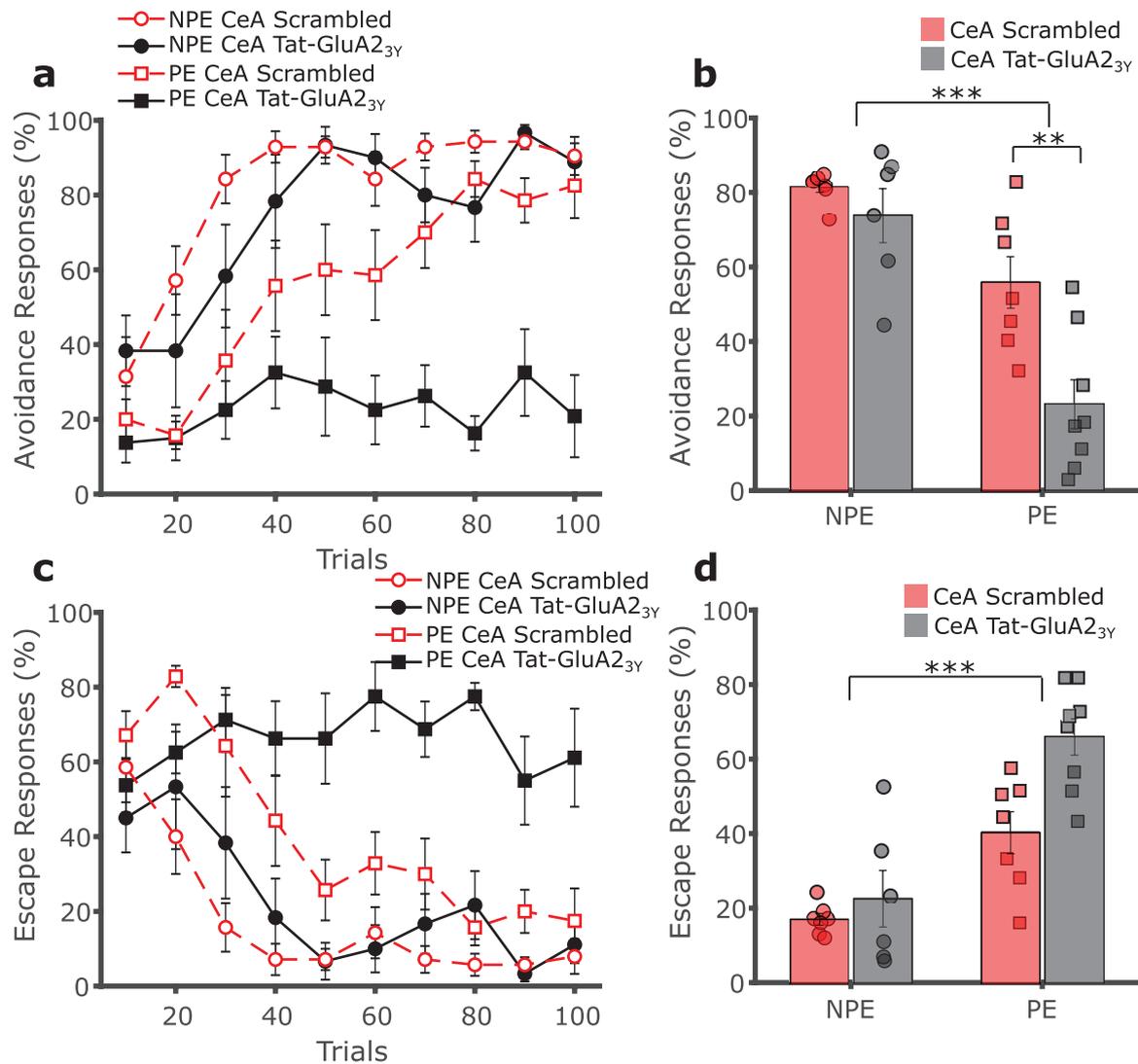


Figure 6. Central nucleus of the amygdala (CeA) administration of Tat-GluA23Y potentiates latent inhibition of 2-way avoidance. (A) Tat-GluA23Y (0.5 μ L/hemisphere, 22.5 pmol/0.5 μ L, IC, black, solid lines) or scrambled control peptide (red, dashed lines) was administered into the CeA of rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls. Intra-CeA Tat-GluA23Y markedly strengthened the LI effect, by further impairing avoidance responding in PE rats (squares) relative to rats administered a scrambled control peptide (red, dashed lines), while having no effect in the NPE group (circles). (B) Overall avoidance percentage indicated a significant reduction of avoidances in intra-CeA Tat-GluA23Y-treated PE rats compared with the NPE group and the corresponding scrambled control, indicating a potentiation of LI. (C) Escape responses progressively declined over trials, with fewer escapes in NPE vs PE rats, while intra-CeA GluA23Y treatment tended to elevate escapes. (D) Overall escape responses percentage was significantly lower in PE vs NPE rats, while intra-CeA Tat-GluA23Y treatment increased escapes regardless of preexposure status. ** $P < .01$, *** $P < .001$, error bars represent SEM.

specifically impairs GluN2B-dependent LTD in vivo (Liu et al., 2004; Fox et al., 2006; Ge et al., 2010), did not recapitulate the potentiated LI effect observed with the Tat-GluA_{23Y} peptide. Considering that systemically induced blockade of glutamatergic transmission using the non-selective NMDA-R blocker MK-801 prior to conditioning potentiates LI (Gaisler-Salomon and Weiner, 2003; Weiner, 2003; Gaisler-Salomon et al., 2008), these results suggest that GluN2B-independent signaling may be involved in both the observed LTD effect on LI as well as the effect of glutamatergic drugs. The possibility remains that localized micro-injections of Ro25-6981 targeting GluN2B-dependent LTD within a specific region of interest are required to affect LI. Therefore, while our findings with the Tat-GluA_{23Y} peptide provide compelling evidence for the role of LTD expression in mediating the observed effect on LI, future studies are warranted to establish which mechanisms of LTD initiation are involved.

The NAC plays a central role in LI as well as in the modulation of LI by dopaminergic drugs (Gray et al., 1997). Although systemic blockade of LTD using Tat-GluA_{23Y} enhances LI similar to the effects of traditional neuroleptics, our microinjection experiments fail to implicate NAC LTD in the LI facilitation. Our microinjections targeted the NAC core, where lesions enhance LI, with limited spread into the shell, where lesions disrupt LI. It is possible that incomplete LTD blockade in the shell accounts for the failure of our intra-NAC microinjections to modulate LI (Weiner et al., 1996; Jongen-Rêlo et al., 2002; Gal et al., 2005). Brain regions that provide afferent projections to the NAC are also implicated in LI, including the mPFC (George et al., 2010; Lingawi et al., 2016) and BLA (Weiner et al., 1995; Coutureau et al., 2001; Schiller and Weiner, 2004). We failed to observe an effect of intra-mPFC injection of the Tat-GluA_{23Y} peptide on LI. We targeted the dorsal aspect of mPFC (anterior cingulate-prelimbic

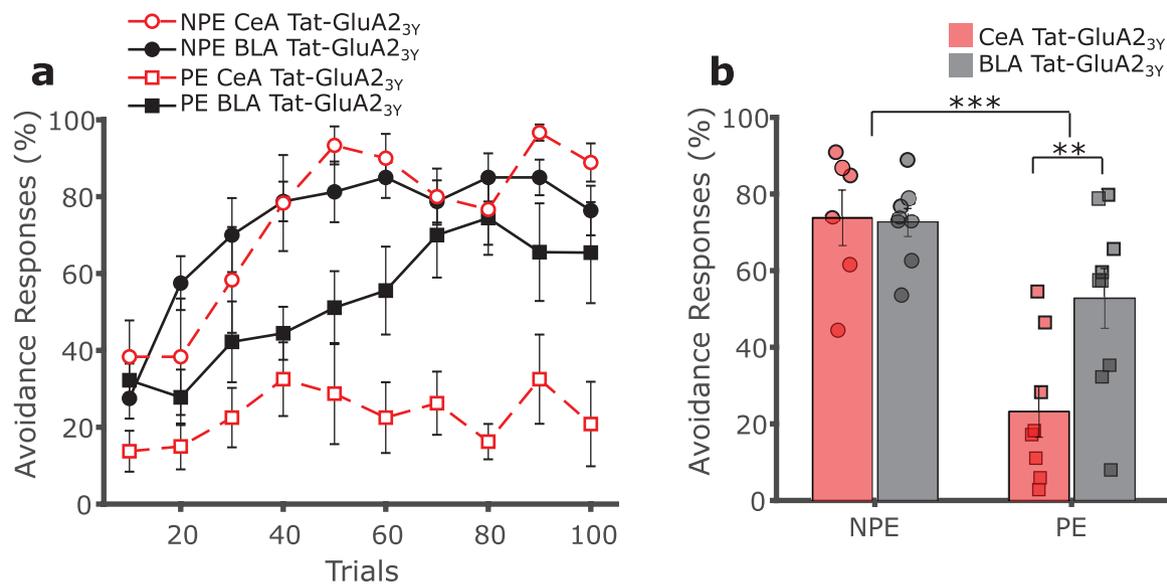


Figure 7. Basolateral amygdala administration of Tat-GluA23Y did not recapitulate the effect on LI observed following CeA administration. (A) Tat-GluA23Y (0.5 μ L/hemisphere, 22.5 pmol/0.5 μ L, IC) was administered into the CeA (red, dashed lines) or BLA (black, dashed lines) to rats prior to conditioning. Rats pre-exposed (PE, squares) to the CS avoided significantly less than non-preexposed (NPE, circles) controls. Intra-BLA Tat-GluA23Y administration had no effects on avoidance responding and thus failed to recapitulate the significant effect on LI observed following intra-CeA administration. This confirmed the CeA as the locus of action Tat-GluA23Y, excluding spill over to the BLA. (B) Overall avoidance percentage indicated a potentiation of LI in intra-CeA but not intra-BLA Tat-GluA23Y-treated rats. ** $P < .01$, *** $P < .001$, error bars represent SEM.

border), which, combined with the volume delivered (0.75 μ L), likely spared ventral mPFC (infralimbic cortex). As ventral mPFC lesions may affect LI (George et al., 2010; Lingawi et al., 2016), a potential role for LTD in specific subregions of the mPFC remains plausible and warrants further investigation.

Administration of the GluA_{23Y} peptide to block LTD within the intra-CeA recapitulated the robust conditioning impairment in CS-PE groups following its systemic administration. This finding, in turn, suggests that LTD in the central amygdala may be a key mediator of cognitive flexibility that enables previously irrelevant stimuli to acquire significance through reinforcement. The CeA is the principal output structure of the amygdala and is required for expression of fear and defensive behaviors to threatening stimuli (LeDoux and Phillips, 1992; Gozzi et al., 2010; Haubensak et al., 2010; Steinberg et al., 2020). Accumulating evidence supports a role for the CeA in both aversive and appetitive emotional learning and as a critical site of plasticity in some forms of Pavlovian learning (Samson and Paré, 2005; Samson et al., 2005; Tillman et al., 2018; Steinberg et al., 2020).

The CeA consists primarily of GABAergic neurons organized into an intricate microcircuit that controls the expression of conditioned fear as an active or passive response (Ciochi et al., 2010; Gozzi et al., 2010; Haubensak et al., 2010; Li et al., 2013; Fadok et al., 2017; Yu et al., 2017). As this GABAergic neuronal circuitry does not undergo GluA2-dependent LTD, we propose that an excitatory input to a subpopulation of CeA neurons is initially potentiated during CS pre-exposure and must then undergo LTD during subsequent conditioning. One candidate might be threat-encoding excitatory inputs from the BLA that synapse onto CeA interneurons, which in turn initiate behavioral responses via inhibitory action onto downstream targets (Babaev et al., 2018). Consideration should also be given to a subpopulation of PKC+ GABAergic cells within the lateral region of the CeA as a possible target of these inputs, as these neurons

cease to display action potentials during acquisition of conditioned fear (Haubensak et al., 2010).

Our data suggest that PE rats in the control and ineffective treatment conditions express both the CS-no event and CS-US associations, whereas PE rats treated with either systemic or intra-CeA administration of the Tat-GluA_{23Y} peptide remain exclusively under the behavioral control of the CS-no event association. Building on Weiner's competition theory of LI, which emphasizes involvement of opposing processes between 2 conflicting associations competing for behavioral expression/retrieval, LTD in the CeA may mediate active inhibition of neural circuitry that encodes the original stimulus-no event association. This, in turn, would permit the formation of a new association between this CS and reinforcement. In the absence of such an LTD mechanism to inhibit a prior association with the same CS, as in the presence of the interference peptide, the initial association would effectively compete with the establishment of a new more relevant association. Through its interaction with the midbrain dopamine system (Ahn and Phillips, 2002; Steinberg et al., 2020), the CeA has also been linked to enhanced attention to cues following changes in their predictive value (Holland and Gallagher, 1993a, 2006; Fudge and Haber, 2000; Lee et al., 2006; Tillman et al., 2018). Accordingly, in the LI paradigm, CeA LTD may also prevent enhancement of attention to changes in stimulus salience required for successful conditioning, consistent with the acquisition-failure theory (Lubow et al., 1976; Pearce and Hall, 1980). Thus, modulation of synaptic plasticity in the CeA may have far-reaching consequences for behavioral responses to threat under changing circumstances.

The LI paradigm used here involves the acquisition of an active response to an aversive contingency. LI effects are observed in both passive fear paradigms and appetitive paradigms; therefore, further studies are required to determine whether the

present finding generalizes to these circumstances. Although the CeA is implicated in certain forms of conditioned appetitive behavior (Hall et al., 2001; Holland and Gallagher, 2003; Steinberg et al., 2020), neurotoxic lesions of the CeA failed to modulate LI in an appetitive procedure (Holland and Gallagher, 1993b). As the neural circuitry mediating conditioned fear differs from that subserving the effects of LI on appetitive learning, it remains to be determined whether disruption of LTD in the appropriate synapses would affect cognitive flexibility required for adaptation of appetitive learning.

Interest in LI gains added significance through its link to schizophrenia and related disorders, giving rise to the “2-headed” LI model of schizophrenia (Weiner, 2003; Weiner and Arad, 2009). On one hand, LI deficits are observed in pre-clinical models of schizophrenia symptoms, canonically induced by amphetamine administration, with disrupted context processing highlighted as a key feature (Weiner, 1990; Gray et al., 1991). Similarly, the clinical data generally support reduced LI in acute, non-/recently medicated, positive-symptom schizophrenic patients compared with healthy controls (Lubow, 2010; Lubow and Weiner, 2010b). On the other hand, LI perseveration, or the inability to update behavior based on changing relationships between stimuli and outcomes, is a core disturbance in chronic schizophrenia (Gal et al., 2009; Granger et al., 2016), implying that impaired LTD may be involved. Furthermore, the abnormally persistent LI observed in chronic schizophrenia patients may account for their impaired attentional set shifting, a form of disrupted cognitive flexibility (Weiner, 2003). LI abnormalities in schizophrenia appear to depend on the state of the disorder (acute vs chronic), providing clinical context for our findings of potentiated LI following LTD blockade, with implications for the positive, negative, and cognitive symptoms of schizophrenia (Weiner, 2003).

Finally, the present findings are consistent with preclinical and clinical studies that link dysfunction within the glutamatergic system and aberrant synaptic plasticity to schizophrenia. As mentioned, NMDA receptor antagonists, which induce persistent LI and model aspects of schizophrenia in rodents, support the hypo-glutamatergic hypothesis and the presence of impaired plasticity in schizophrenia (Gaisler-Salomon and Weiner, 2003; Weiner, 2003; Gaisler-Salomon et al., 2008). Consistent with this hypothesis, noninvasive human brain stimulation studies report reduced LTP and LTD in the motor cortex of schizophrenia patients (Hasan et al., 2012; Bhandari et al., 2016). Modulation of LTD provides a potent mechanism for targeting aberrant LI and deserves consideration as a potential therapeutic strategy for key symptoms in schizophrenia arising from abnormal stimulus processing. In summary, the present findings have relevance both for our mechanistic understanding of LI and its alteration in pathological states such as schizophrenia, while further elucidating the role of LTD in learning and memory.

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Statement of Interest

Y.T.W. and A.G.P. declare a patent related to glutamate receptor function (A Peptide that Specifically Blocks Regulated AMPA Receptor Endocytosis and Hippocampal CA1 Long-term Depression; European 04789721.0, and United States 13/066,700). A.G.P. also declares a patent related to the use of d-govadine to enhance dopamine function in the prefrontal cortex (Tetrahydroprotoberbine compounds and uses thereof in the treatment of neurological, psychiatric and neurodegenerative diseases; US20150306092). A.G.P. also holds shares in Resilience Biosciences Inc., Canada. Other authors report no other biomedical financial interests or potential conflicts of interest.

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